

We claim:

Su1a3 >

1. A dual-specificity antibody, or antigen-binding portion thereof, that specifically binds interleukin-1 α and interleukin-1 β .
- 5 2. The dual-specificity antibody of claim 1, or antigen-binding portion thereof, which binds interleukin-1 α with a k_{off} rate constant of 0.1s^{-1} or less, as determined by surface plasmon resonance, or which inhibits the activity of interleukin-1 α with an IC_{50} of $1 \times 10^{-5}\text{ M}$ or less.

- 10 3. The dual-specificity antibody of claim 1, or antigen-binding portion thereof, which binds interleukin-1 β with a k_{off} rate constant of 0.1s^{-1} or less, as determined by surface plasmon resonance, or which inhibits the activity of interleukin-1 β with an IC_{50} of $1 \times 10^{-5}\text{ M}$ or less.

Su1a4 >

4. A method of obtaining a dual-specificity antibody that specifically binds interleukin-1 α and interleukin-1 β , the method comprising:
 - 15 providing an antigen that comprises a common structural feature of IL-1 α and IL-1 β ;
 - exposing an antibody repertoire to the antigen; and
 - selecting from the repertoire an antibody that specifically binds IL-1 α and IL-1 β to thereby obtain the dual specificity antibody.

- 20 5. The method of claim 4, wherein the antigen is designed based on a contiguous topological area of identity between IL-1 α and IL-1 β .

6. The method of claim 5, wherein the antigen comprises the amino acid sequence NEAQNITDF (SEQ ID NO: 1) or dNdEdAdQNITDF.

7. The method of claim 4, wherein the antigen is designed based on 25 structurally mimicking a loop of a common fold of IL-1 α and IL-1 β .

8. The method of claim 7, wherein the antigen is a cyclic peptide comprising the amino acid sequence Cyclo-MAFLRANQNNGKISVAL(PG) (SEQ ID NO: 2).

Su1a5 >

9. The method of claim 4, wherein the antigen is designed based on splicing 30 together overlapping portions of IL-1 α and IL-1 β to create a hybrid molecule.

10. The method of claim 9, wherein the antigen comprises the amino acid sequence TKGGQDITDFQILENQ (SEQ ID NO: 3).

11. The method of claim 4, wherein the antigen comprises the amino acid sequence

5 APVRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMGAYKSSKD
DAKITVILGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKMEKRFVFNKIEI
NNKLEFESAQFPNWYISTSQAENMPVFLGGTKGGQDITDFTMQFVSS
(SEQ ID NO: 4).

12. The method of claim 4, wherein the antibody repertoire is exposed to the 10 antigen *in vivo* by immunizing an animal with the antigen.

13. The method of claim 12, which further comprises preparing a panel of hybridomas from lymphocytes of the animal and selecting a hybridoma that secretes an antibody that specifically binds IL-1 α and IL-1 β .

14. The method of claim 12, wherein the animal is selected from the group 15 consisting of mice, rats, rabbits and goats.

15. The method of claim 12, wherein the animal is a knockout mouse deficient for IL-1 α , IL-1 β or both IL-1 α and IL-1 β .

16. The method of claim 12, wherein the animal is a mouse that is transgenic 20 for human immunoglobulin genes such that the mouse makes human antibodies upon antigenic stimulation.

17. The method of claim 12, wherein the animal is a mouse with severe combined immunodeficiency (SCID) that has been reconstituted with human peripheral blood mononuclear cells or lymphoid cells or precursors thereof.

18. The method of claim 12, wherein the animal is a mouse that has been 25 treated with lethal total body irradiation, followed by radioprotection with bone marrow cells of a severe combined immunodeficiency (SCID) mouse, followed by engraftment with functional human lymphocytes or precursors thereof.

19. The method of claim 4, wherein the antibody repertoire is exposed to the antigen *in vitro* by screening a recombinant antibody library with the antigen.

30 20. The method of claim 19, wherein the recombinant antibody library is expressed on the surface of bacteriophage.

21. The method of claim 19, wherein the recombinant antibody library is expressed on the surface of yeast cells.

22. The method of claim 19, wherein the recombinant antibody library is expressed on the surface of bacterial cells.

5 23. The method of claim 19, wherein the recombinant antibody library is expressed as RNA-protein fusions.

24. The method of claim 19, wherein the recombinant antibody library is a scFv library or a Fab library.

10 25. The method of claim 4, wherein the antibody repertoire is exposed to the antigen by *in vivo* immunization of an animal with the antigen, followed by *in vitro* screening of a recombinant antibody library prepared from lymphoid cells of the animal with the antigen.

15 26. The method of claim 4, wherein the antibody repertoire is exposed to the antigen by *in vivo* immunization of an animal with the antigen, followed by *in vitro* affinity maturation of a recombinant antibody library prepared from lymphoid cells of the animal.

20 27. The method of claim 4, wherein the antibody repertoire is exposed to the antigen by *in vivo* immunization of an animal with the antigen, followed by selection of single cells secreting antibodies that bind the antigen and recovery of heavy- and light-chain variable region cDNAs from the single cells.

28. The method of claim 4, wherein the dual-specificity antibody is a fully human antibody.

29. The method of claim 4, wherein the dual-specificity antibody is a chimeric antibody.

25 30. The method of claim 4, wherein the dual-specificity antibody is a CDR-grafted antibody.

Sub A6 ~~31. A dual-specificity antibody, or antigen-binding portion thereof, that specifically binds interleukin-1 α and interleukin-1 β obtainable by the method of claim 4.~~

30 32. A method of obtaining a dual-specificity antibody that specifically binds two different but structurally related molecules, the method comprising:

providing an antigen that comprises a common structural feature of the two different but structurally related molecules;

exposing an antibody repertoire to the antigen; and

selecting from the repertoire an antibody that specifically binds the two

- 5 different but structurally related molecules to thereby obtain the dual specificity antibody.

33. The method of claim 32, wherein the antigen is designed based on a contiguous topological area of identity between the two different but structurally related molecules.

- 10 34. The method of claim 33, wherein the two different but structurally related molecules are proteins and the antigen is a peptide comprising an amino acid sequence of the contiguous topological area of identity between the two proteins.

35. The method of claim 32, wherein the antigen is designed based on structurally mimicking a loop of a common fold of the two different but structurally related molecules.

- 15 related molecules.

36. The method of claim 35, wherein the two different but structurally related molecules are proteins and the antigen is a cyclic peptide that structurally mimics a loop of a common fold of the two proteins.

37. The method of claim 32, wherein the antigen is designed based on

- 20 splicing together overlapping portions of the two different but structurally related molecules to create a hybrid molecule.

38. The method of claim 37, wherein the two different but structurally related molecules are proteins and the antigen is a hybrid peptide made by splicing together overlapping amino acid sequences of the two proteins.

- 25 39. The method of claim 32, wherein the antigen is one of the two different
but structurally related molecules.

40. The method of claim 32, wherein the antibody repertoire is exposed to the antigen *in vivo* by immunizing an animal with the antigen.

41. The method of claim 40, which further comprises preparing a panel of

- 30 hybridomas from lymphocytes of the animal and selecting a hybridoma that secretes an antibody that specifically binds the two different but structurally related molecules.

42. The method of claim 40, wherein the animal is selected from the group consisting of mice, rats, rabbits and goats.

43. The method of claim 40, wherein the animal is a knockout mouse deficient for an endogenous version of the antigen.

5 44. The method of claim 40, wherein the animal is a mouse that is transgenic for human immunoglobulin genes such that the mouse makes human antibodies upon antigenic stimulation.

10 45. The method of claim 40, wherein the animal is a mouse with severe combined immunodeficiency (SCID) that has been reconstituted with human peripheral blood mononuclear cells or lymphoid cells or precursors thereof.

46. The method of claim 40, wherein the animal is a mouse that has been treated with lethal total body irradiation, followed by radioprotection with bone marrow cells of a severe combined immunodeficiency (SCID) mouse, followed by engraftment with functional human lymphocytes.

15 47. The method of claim 32, wherein the antibody repertoire is exposed to the antigen *in vitro* by screening a recombinant antibody library with the antigen.

48. The method of claim 47, wherein the recombinant antibody library is expressed on the surface of bacteriophage.

20 49. The method of claim 47, wherein the recombinant antibody library is expressed on the surface of yeast cells.

50. The method of claim 47, wherein the recombinant antibody library is expressed on the surface of bacterial cells.

51. The method of claim 47, wherein the recombinant antibody library is expressed as RNA-protein fusions.

25 52. The method of claim 47, wherein the recombinant antibody library is a scFv library or a Fab library.

53. The method of claim 32, wherein the antibody repertoire is exposed to the antigen by *in vivo* immunization of an animal with the antigen, followed by *in vitro* screening of a recombinant antibody library prepared from lymphoid cells of the animal 30 with the antigen.

54. The method of claim 32, wherein the antibody repertoire is exposed to the antigen by *in vivo* immunization of an animal with the antigen, followed by *in vitro*

affinity maturation of a recombinant antibody library prepared from lymphoid cells of the animal.

55. The method of claim 32, wherein the antibody repertoire is exposed to the antigen by *in vivo* immunization of an animal with the antigen, followed by selection of 5 single cells secreting antibodies that bind the antigen and recovery of heavy- and light-chain variable region cDNAs from the single cells.

56. The method of claim 32, wherein the dual-specificity antibody is a fully human antibody.

57. The method of claim 32, wherein the dual-specificity antibody is a 10 chimeric antibody.

58. The method of claim 32, wherein the dual-specificity antibody is a CDR-grafted antibody.

59. A dual-specificity antibody, or antigen-binding portion thereof, obtainable by the method of claim 32.

15 60. A method of detecting IL-1 α or IL-1 β in a biological sample or tissue comprising contacting the biological sample or tissue suspected of containing IL-1 α or IL-1 β with the dual-specificity antibody, or antigen-binding portion thereof, of claim 1 and detecting IL-1 α or IL-1 β in the biological sample or tissue.

61. The method of claim 60, wherein IL-1 α or IL-1 β is detected for 20 diagnostic purposes.

62. The method of claim 60, wherein the biological sample is an *in vitro* sample.

63. The method of claim 60, wherein the tissue is located *in vivo* in a subject and the method comprises *in vivo* imaging of the tissue.

25 64. A method of inhibiting IL-1 α or IL-1 β activity comprising contacting IL-1 α or IL-1 β with the dual-specificity antibody, or antigen-binding portion thereof, of claim 1 such that the activity of IL-1 α or IL-1 β is inhibited.

65. The method of claim 64, wherein IL-1 α or IL-1 β activity is inhibited *in vitro*.

30 66. A method of treating an interleukin-1-related disorder comprising administering to a subject suffering from an interleukin-1-related disorder the dual-

specificity antibody, or antigen-binding portion thereof, of claim 1, such that the subject is treated for the interleukin-1-related disorder.

67. The method of claim 66, wherein the IL-1-related disorder is an inflammatory disorder.

5 68. The method of claim 66, wherein the IL-1-related disorder is an autoimmune disorder.

69. The method of claim 66, wherein the IL-1-related disorder is selected from the group consisting of rheumatoid arthritis, Crohn's disease, multiple sclerosis, insulin dependent diabetes, mellitus and psoriasis.

10 70. A method of making an antibody or an antigen binding portion thereof library comprising the steps of:

a) obtaining a recombinant heavy chain or an antigen binding portion thereof library A from an antibody repertoire resulting from exposure to a first antigen;

15 b) obtaining a recombinant light chain or an antigen binding portion thereof library B from an antibody repertoire resulting from exposure to the first antigen;

c) obtaining a recombinant heavy chain or an antigen binding portion thereof library C from an antibody repertoire resulting from exposure to a second antigen;

20 d) obtaining a recombinant light chain or an antigen binding portion thereof library D from an antibody repertoire resulting from exposure to the second antigen; and

e) combining the recombinant heavy chain or an antigen binding portion thereof library A with the recombinant light chain or an antigen binding portion thereof library D to obtain an antibody or an antigen binding portion thereof library X and/or combining the recombinant heavy chain or an antigen binding portion thereof library C with the recombinant light chain or an antigen binding portion thereof library B to obtain an antibody or an antigen binding portion thereof library Y.

25 71. A method of making an antibody or an antigen binding portion thereof library according to claim 70 further comprising the step of combining

- the antibody or an antigen binding portion thereof library X with the antibody or an antigen binding portion thereof library Y to obtain an antibody or an antigen binding portion thereof library Z.
72. The antibody or an antigen binding portion thereof library X made according to the method of claim 70.
- 5 73. The antibody or an antigen binding portion thereof library Y made according to the method of claim 70.
74. The antibody or an antigen binding portion thereof library Z made according to the method of claim 71.
- 10 75. A method of making a dual specific antibody or an antigen binding portion thereof comprising the steps of:
- a) obtaining a recombinant heavy chain or an antigen binding portion thereof library A from an antibody repertoire resulting from exposure to a first antigen;
- 15 b) obtaining a recombinant light chain or an antigen binding portion thereof library B from an antibody repertoire resulting from exposure to the first antigen;
- c) obtaining a recombinant heavy chain or an antigen binding portion thereof library C from an antibody repertoire resulting from exposure to a second antigen;
- 20 d) obtaining a recombinant light chain or an antigen binding portion thereof library D from an antibody repertoire resulting from exposure to the second antigen;
- e) combining the recombinant heavy chain or an antigen binding portion thereof library A with the recombinant light chain or an antigen binding portion thereof library D to obtain an antibody or an antigen binding portion thereof library X and/or combining the recombinant heavy chain or an antigen binding portion thereof library C with the recombinant light chain or an antigen binding portion thereof library B to obtain an
- 25 antibody or an antigen binding portion thereof library Y; and
- f) selecting from the antibody or an antigen binding portion thereof library X and/or antibody or an antigen binding portion thereof library Y
- 30
- PCT/GB2008/002260

- an antibody or an antigen binding portion thereof that binds both the first and the second antigen.
76. The dual specific antibody made by the method of claim 75.
77. A method of making a dual specific antibody or an antigen binding portion thereof comprising the steps of:
- 5 a) obtaining a recombinant heavy chain or an antigen binding portion thereof library A from an antibody repertoire resulting from exposure to a first antigen;
- 10 b) obtaining a recombinant light chain or an antigen binding portion thereof library B from an antibody repertoire resulting from exposure to the first antigen;
- 15 c) obtaining a recombinant heavy chain or an antigen binding portion thereof library C from an antibody repertoire resulting from exposure to a second antigen;
- 20 d) obtaining a recombinant light chain or an antigen binding portion thereof library D from an antibody repertoire resulting from exposure to the second antigen;
- 25 e) combining the recombinant heavy chain or an antigen binding portion thereof library A with the recombinant light chain or an antigen binding portion thereof library D to obtain an antibody library X and/or combining the recombinant heavy chain or an antigen binding portion thereof library C with the recombinant light chain or an antigen binding portion thereof library B to obtain an antibody or an antigen binding portion thereof library Y;
- 30 f) combining the antibody or an antigen binding portion thereof library X with the antibody library or an antigen binding portion thereof Y to obtain an antibody or an antigen binding portion thereof library Z; and
- g) selecting from the antibody or an antigen binding portion thereof library Z an antibody or an antigen binding portion thereof that binds both the first and the second antigen.
78. The dual specific antibody or an antigen binding portion thereof made by the method according to claim 77.

- TOP SECRET//~~REF ID: A60~~
79. The method according to claims 70 wherein the first and second antigen is each independently selected from the group consisting of proteins, polypeptides and peptides provided that the first and second antigens are not the same.
- 5 80. The method of claim 79, wherein the proteins, polypeptides and peptides are secreted proteins or surface receptors.
81. The method of claim 80, wherein the secreted protein is selected from the group consisting of an IFN, a TNF, an Interleukin, IP-10, PF4, a GRO, 9E3, EMAP-II, a CSF, an FGF, and a PDGF.
- 10 82. The method according to claim 81, wherein the first antigen is IL-1 α and the second antigen is IL-1 β .
83. The nucleotide sequence encoding each member of the antibody or an antigen binding portion thereof library or libraries according to claims 72.
84. The nucleotide sequence encoding the dual specific antibody or an antigen binding portion thereof according to claims 76.
- 15 85. A vector comprising the nucleotide sequence encoding a member of the antibody or an antigen binding portion thereof library or libraries of claims 72.
86. A vector comprising the nucleotide sequence encoding the dual specific antibody or an antigen binding portion thereof according to claims 76.
- 20 87. A host cell transfected with the vector of claim 85.
88. A host cell transfected with the vector of claim 86.

Adha 